Induced pluripotent stem cells reprogramming: Epigenetics and applications in the regenerative medicine

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Summary

Induced pluripotent stem cells (iPSCs) are somatic cells reprogrammed into an embryonic-like pluripotent state by the expression of specific transcription factors. iPSC technology is expected to revolutionize regenerative medicine in the near future. Despite the fact that these cells have the capacity to self-renew, they present low efficiency of reprogramming. Recent studies have demonstrated that the previous somatic epigenetic signature is a limiting factor in iPSC performance. Indeed, the process of effective reprogramming involves a complete remodeling of the existing somatic epigenetic memory, followed by the establishment of a "new epigenetic signature" that complies with the new type of cell to be differentiated. Therefore, further investigations of epigenetic modifications associated with iPSC reprogramming are required in an attempt to improve their self-renew capacity and potency, as well as their application in regenerative medicine, with a new strategy to reduce the damage in degenerative diseases. Our review aimed to summarize the most recent findings on epigenetics and iPSC, focusing on DNA methylation, histone modifications and microRNAs, highlighting their potential in translating cell therapy into clinics.

Keywords: induced pluripotent stem cells, regenerative medicine, cell reprogramming, epigenetics, histones, microRNAs.

Introduction

Human embryonic stem cells have great potential for self-renewal and the ability to differentiate into all tissues of the body (except embryonic attachments),[1] forming an important source of material for regenerative medicine and cell therapy. However, the use of embryonic stem cells is limited by ethical and religious conflicts, as well as immunological incompatibility.[2] In order to reduce the damage caused by degenerative diseases, different strategies are being used in an attempt to optimize the use of embryonic stem cells.

The first strategy used was that of somatic cell nuclear content (SCNT) for unfertilized and enucleated oocytes.[3] However, the yield of this technique is still very low and the cells obtained can present phenotypic and gene expression abnormalities.[4] Another strategy is the fusion of somatic cells with embryonic cells, reprogramming their genome.[5] However, although fusion-induced reprogramming is very efficient (about 95%), the resulting hybrid cells lack therapeutic potential due to their tetraploidy, as well as the presence of exogenous genes from the pluripotent cells used in the fusion.[5] Therefore, a search for new strategies for the efficient use of cells with an embryonic profile is still needed.

In 2006 Takahashi and Yamanaka managed to induce pluripotency in mouse fibroblasts (MEF) from the expression of four embryonic transcription factors (Oct4, Sox2, Klf4, c-Myc), currently known as OSKM or "Yamanaka factors." These cells were called induced pluripotent stem cells (iPSCs).[6] The iPSC are similar to embryonic cells in terms of morphology, gene expression, differentiation status and epigenetic pattern, both in culture, as well as in vivo.[7] This mechanism of reprogramming somatic cells into embryonic stem cells resulted in the Nobel Prize in

medicine for Yamanaka in 2012, shared with John b. Gurdon.[8] Since then, several surveys have been developed to explore this technology. In fact, several researchers were able to reprogram somatic cells (postmitotic) into iPSC using the aforementioned strategy.[9-11]

The advantage of using this method is that it allows the derivation of pluripotent cells from the donor, reducing the risk of rejection by the immune system. In addition, this method provides a platform to study the molecular mechanisms of genetic and chronic diseases, minimizing the ethical, religious and political conflicts and opening up new perspectives for regenerative medicine. However, although cell identity can be modified by the ectopic expression of transcription factors, the efficiency of reprogramming remains low (0.1 to 3%) and its cost is high.[11,12]

The low reprogramming efficiency of iPSC is associated with residual epigenetic memory of the tissue from which they were derived, which complicates the reprogramming process. Recent studies show that despite iPSC sharing common characteristics of pluripotency and self-renewal capacity, these cells still retain an epigenetic memory.[13-15] In addition, there is evidence that the reprogramming process involves complete remodeling of the existing somatic epigenetic memory, followed by the establishment of a new "epigenetic signature" that conforms to the type of cell to be differentiated.[16] Therefore, the epigenetic memory becomes a barrier in the process of cellular reprogramming. This fact highlights the need for new studies investigating the epigenetic changes associated with cellular reprogramming in an attempt to improve the efficiency and effectiveness of the iPSC created, as well as their clinical application. As such, our review aimed to gather information about the epigenetic factors (DNA methylation, changes to histones and microRNAs) associated with iPSC reprogramming efficiency. In addition, we have brought together the clinical studies using iPSC as cell therapy.

EPIGENETICS AND iPSC

Waddington was the first researcher to use the term epigenetics in 1942 to explain how the genome interacts with the environment during the development process.[17] Therefore, any reversible and inheritable change in the functional genome that does not alter the sequence of DNA nucleotides refers to epigenetics.[18] Several pathologies are associated with epigenetic changes.[19-22]

The reprogramming efficiency of iPSC is also directly related to epigenetic changes such as DNA methylation and the epigenetic memory of the source cells.[23] The iPSC reprogramming process can be divided into three distinct phases, called pre-iPSC, intermediate and full reprogramming. The reprogramming process is extremely slow, with low efficiency (0.1 to 3%) and high cost,[11,12] and depends on suitable levels of gene expression in each phase and specific epigenetic changes.[24] Djuric and Ellis compared the epigenetic changes that occur during the reprogramming process with a "seven headed dragon," where a series of changes is necessary for efficient reprogramming, namely: 1) Endogenous reactivation of genes related to cell pluripotency, Nanog and Oct4; 2) Chromatin changes, such as trimethylation in H3K27 and changes in H3K4; 3) Hypomethylation of heterochromatin; 4) Reactivation of the inactive X chromosome; 5) Maintenance of DNA methylation marks; 6) Silencing the retrovirus that induces pluripotency; and, finally, 7) Two- or three-dimensional chromatin changes and location of nuclear subdomains.[24] Therefore, the control of epigenetic factors during reprogramming may improve the induction of iPSC and their efficiency.[25]

EPIGENETIC CHANGES IN iPSC REPROGRAMMING

DNA methylation in iPSC reprogramming

DNA methylation is an epigenetic mechanism involved in many important cellular processes such as cell proliferation and differentiation, transcriptional repression, genomic imprinting, organization of chromatin and inactivation of the X chromosome.[26] Thus, changes in the DNA methylation profile are associated with the appearance of many degenerative diseases.

Studies show that DNA methylation is considered a crucial epigenetic barrier in the reprogramming of iPSC.[27-31] For the expression of genes essential for reprogramming, such as Oct3/4 and Nanog, demethylation of cytosines is necessary in the respective promoter regions.[32] Thus, inhibition of methylation by enzymes or interfering RNA may be an option to improve this process.[33] Mikkelsen et al. showed that the use of 5-azacytidine (AZA) inhibits the enzyme DNA methyltransferase 1, assisting in DNA demethylation, which may favor the reprogramming of iPSC.[33] Another study noted an improvement in reprogramming efficiency using DNA hydroxylase (Tet1), an enzyme that is able to oxidize 5-methylcytosine and, after subsequent replications, promotes DNA demethylation by reactivating Oct4 gene transcription, favoring the reprogramming process.[31] In addition to the use of AZA and the enzyme Tet1, other researchers have used ascorbic acid (vitamin C). Vitamin C acts as a cofactor, stimulating hypomethylation and consequently increasing the activity of histone H3K36 demethylase. This process results in an improvement of cellular reprogramming efficiency.[34-36]

The aforementioned studies demonstrate the importance of DNA demethylation in cell reprogramming. Several studies have attempted to improve these and other epigenetic mechanisms in order to improve both the quality and efficiency of iPSC reprogramming. Another relevant topic for such improvement is the change in histones during the reprogramming process.

Changes to histones in iPSC reprogramming

Histones are basic proteins rich in lysine and may suffer several epigenetic changes. Most of these modifications happen in the N-terminal region of the histone, with the exception of ubiquitination, which occurs in the C-terminal region of H2A and H2B.[37] Epigenetic modifications to histones may either promote or inhibit gene transcription by changing the level of chromatin folding.[38,39]

Taking into consideration the epigenetic changes in histones in iPSC, H3 is the histone researched the most, as it is directly related to genes expressed during embryonic development, such as Oct3/4, Sox2 and Nanog. It has already been demonstrated that methylation of H3K27 is associated with the suppression of various genes, and that persistent trimethylation of the lysine 27 of histone 3 (H3K27me3) blocks reprogramming by repressing the chromatin region associated with the target genes of the stem cells. However, the methylation of H3K4 is associated with the activation of different embryonic genes.[24] In an attempt to improve the performance of iPSC and reach the ideal conditions for the induction of pluripotency, by reducing the "epigenetic memory" in somatic cells, different strategies that directly or indirectly affect the methylation/acetylation of H3 have been used.[12] Several researchers have demonstrated that it is possible to perform the induction of pluripotency without the use of Yamanaka factors, using only chemical compounds/molecules that interfere with the enzymes that control the chromatin structure.[40]

Recently, Rais et al. showed that the inhibition of Mbd3 – a subunit of the NuRD complex responsible for the deacetylation of histones, remodeling of nucleosomes and gene expression inhibition – is able to reactivate the genes expressed during embryonic development and improve the efficiency of iPSC reprogramming by almost 90%, both in human as well as mouse cells.[41]

Many other strategies have been tested in order to improve the iPSC reprogramming process, such as the use of small molecules like Forskolin (FSK),[42] BIX-01294,[43] valproic acid (VPA – HDAC histone deacetylase inhibitor)[44] and vitamin C.[45] Therefore, the induction of pluripotency of iPSC can only occur with the use of small molecules.[46-48] The authors advocate the use of such because they are not immunogenic, with greater yield and easy production. Thus, the use of chromatin modulators can increase efficiency in the iPSC reprogramming process.[49] To do so, it is necessary to use a small molecule that is able to demethylate the DNA in the promoter region and change specific regions in histones.

MicroRNAs in iPSC reprogramming

MicroRNA or miRNA are important tools for regulating gene expression in post-transcriptional iPSC by promoting pluripotency to modulate the stability of messenger RNA (mRNA) and suppress the signs of differentiation during the self-renewal of embryonic stem cells.

MiRNAs also modulate the signaling cascades that are necessary for maintaining the pluripotent state.[50] Wang et al. noted that the loss of function in the enzyme Dicer and DGCR8, proteins essential for the biogenesis of microRNAs in the embryonic stem cells of mice, present two different phenotypes: 1) reduction in proliferation due to cell cycle arrest in G1; and 2) resistance to differentiation, which reveals a close relationship between microRNAs, differentiation and the pluripotency of cells.[51,52]

MicroRNAs are small non-coding RNA molecules. They have 18-25 nucleotides (nt), and are derived from a larger precursor. The processing of microRNAs occurs as follows:

1. After DNA transcription by RNA polymerase II or III the primary microRNA is formed (pri-miRNA). This may be presented in the shape of a fork. The pri-miRNA first undergoes processing by the enzyme ribonuclease (RNase) nuclear III-like DROSHA. The specificity of the cleavage in this step is guided by the DGCR8 protein, which acts as a "molecular ruler," positioning the DROSHA at a distance of 11 nucleotides from the base of the pri-miRNA loop. After cleavage, a pre-miRNA (precursor miRNA) is released, formed by about 60-70 nt.[53]

2. The pre-miRNA is actively transported to the cytoplasm by exportin-5 (Exp5), when this protein is linked to its Ran-dependent GTP cofactor. In the cytoplasm, it undergoes another cleavage process, where it loses the loop and is reduced to a miRNA duplex approximately 18-25pb in length. This last stage of processing is conducted by DICER, an RNAse-III type enzyme, aided by the TRBP protein.[53]

3. Finally, the RNA-binding protein TRBP recruits a multimeric complex denominated RISC (RNA-induced silence complex), which includes the protein Argonaute 2 (in mammals) as the main component.

Only one of the microRNA duplex strands remains in the RISC complex (guide strand), with the other being degraded (passenger strand). The RISC complex is able to identify and bind to target messengers RNAs in region 3'UTR through complementarity of bases in the "SEED" region of the miRNA (nucleotides 2-8 from the 5' end) in order to inhibit its translation or promote its adenylation and degradation.[53]

MicroRNAs that promote the reprogramming of iPSC

- miR 290-295 (cluster): These constitute more than 70% of the entire population of microRNAs in the embryonic stem cells of mice. miR 291-3 p, miR-294 and miR-295 are part of this cluster and indirectly promote the transition of genes associated with entry into the G1-S phase, blocking *Cdkna* (p21), a suppressor of the Cyclin E/Cdk2 complex, and regulator of the cell cycle. After its transfection into MEFs there is an increase of 0.01-0.05% to 0.1-0.3% in cell reprogramming efficiency.[54]
- miR 302-367 and miR 371-373 (cluster): These miRNAs suppress the expression of MBD2 (methyl-CPG-binding domain protein 2), which works like a demethylase in cells, resulting in increased expression of Nanog and conversion of completely reprogrammed iPSC. They are also able to reduce expression of the inhibitors in the G1-S phase, as well as increasing the kinetics of the mesenchymal-epithelial transition (MET) required for reprogramming.[55,56] Only the use of miR-302a/b/c/d and miR-367 is able to reprogram adult cells, and with greater efficiency, when compared to the Yamanaka method.[57] Data demonstrate that miR-302 in conjunction with Yamanaka factors inhibits NR2F2 (nuclear receptor subfamily 2, group F, member 2) and improves reprogramming efficiency through indirect positive regulation of Oct4.[58]
- miR-200b and -200c, miR-205: These promote MET via signaling of transforming growth factor β (TGF-β) and, in conjunction with the Yamanaka factors, exclude the need for signaling of bone morphogenetic protein (BMP) during the initial reprogramming phase.[59]
- miR-93, miR-106: They suppress the expression of TGF-β and p21, leading to MET and increased proliferation.[60]
- miR-135b: This is highly expressed during the reprogramming process, regulating the expression of TGF-β, IGFBP5 (insulin-like growth factor binding protein 5) and Wisp1 (inducible-signaling pathway protein 1), which are involved in the expression of extracellular matrix genes.[61]

MicroRNAs that are barriers to iPSC reprogramming

- miR Let-7 (cluster): This inhibits the Pou5f1/Oct4, Sox2, Nanog and Tcf3 targets – pluripotency factors – stabilizing a differentiated state. In addition, this miRNA inhibits the translation of CDK4, repressing the transition of the G1-S-phase. MiR Let-7 forms a negative feedback circuit, providing a molecular mechanism that facilitates the decision between self-renewal and differentiation of cells.[50,62]
- miR-34a, miR-34b/c: miR-34a represses the expression of Nanog, Sox2 and c-Myc. Taken in conjunction, miR-34a and miR-34b/c target p53 (tumor suppressor gene), holding an essential role in the containment of somatic reprogramming.[63]

These studies show that miRNAs can be important tools in the mediation of iPSC reprogramming without the need for the ectopic expression of pluripotency induction factors, including OSKM factors. The tables below present a summary of different approaches and their effects on iPSC reprogramming. Table 1 is related to changes in DNA methylation, Table 2 is related to modifications in histones and Table 3 is related to the use of miRNAs. In addition, Figure 1 presents these changes in summarized form.

CLINICAL APPLICATION OF iPSCs
The main discussion about the use of iPSC in regenerative medicine is related to their ability to transform into cancer cells. Incomplete reprogramming of iPSC may be associated with the emergence of various mutations.

In addition to the impact on iPSC reprogramming, OSKM factors are associated with the development of tumors. Oct4 is highly expressed in the cervical cancer cell line. Deletion of Sox2 is associated with regression of melanomas. The Klf4 and c-Myc factors regulate genes involved in cell growth and proliferation.[64] However, over the last 10 years following the discovery of iPSC, this technology has undergone several changes, such as the use of episomal plasmids that are not integrated into the genome, diminishing the carcinogenic potential of these cells.[65] Currently, several methods are used to develop iPSC lines, including the use of plasmids, transposons, adenovirus, Sendai virus, miRNA and chemical compounds, minimizing mutagenic factors. Given these advances, iPSC have been used in pre-clinical tests and clinical trials.

The first transplant of iPSC in humans occurred in Japan in the second half of 2014, in a 70-year-old patient with age-related macular degeneration (AMD). This pa-

TABLE 1 Epigenetic DNA changes.

Authors	Year	Species	Type of cell	Technology	Epigenetic changes	Results
Mikkelsen et al.[33]	2008	Mice	Fibroblasts	5-azacytidine (AZA)	Inhibitor of DNA methyltransferase	Promotes the demethylation of pluripotency genes
Mikkelsen et al.[33]	2008	Mice	Fibroblasts	shRNA/siRNA	Reduced expression of DNMT1 using siRNA/shRNA	Demethylation and complete reprogramming of cells
Popp et al.[28]	2010	Mice	Embryonic	AID	DNA demethylation	Clears all standard demethylation of the genome
Doege et al.[29]	2012	Mice	Fibroblasts	Tet2	Generation of 5-hydroxymethylcytosine (5hmC) through the oxidation of 5mC	Hydroxylation of pluripotency sites (Nanog and Esrrb) favoring a greater number of iPSCs
Costa et al.[30]	2013	Mice	Embryonic	Tet1/Tet2	Physical interaction with Nanog	Hydroxylation of pluripotency sites (Oct4 and Esrrb)
Gao et al.[31]	2013	Mice	Fibroblasts	Tet1	DNA demethylation and activation of Oct4	Better induction of pluripotency

iPSCs: induced pluripotent stem cells.

TABLE 2 Epigenetic changes in histones.

Authors	Year	Species	Type of cell	Technology	Epigenetic changes	Results
Huangfu et al.[44]	2008	Mice	Fibroblasts	VPA/TSA/SAHA	Inhibitor of histone deacetylation	Prevents histone deacetylation, increasing reprogramming efficiency by more than 100X compared to OSKM
Shi et al.[43]	2008	Mice	Fibroblasts	BIX-01294	G9a methyltransferase inhibitor	Reduces histone H3K9 methylation, facilitating transcription. Improves reprogramming efficiency compared to Oct4 and Klf4
Esteban et al.[35]	2010	Humans	Fibroblasts	Vitamin C	Nanog promoter, Oct4/ Histone demethylation	Decreases senescence during reprogramming
Liang et al.[46]	2010	Mice	Fibroblasts	Sodium butyrate	Histone deacetylase (HDAC) inhibitor	Facilitates reprogramming mediated by c-Myc
Liang et al.[48]	2012	Mice	Fibroblasts	Lentivirus increasing the expression of Kdm2b	Activation of genes in the initial phase of reprogramming	Decreased H3K36me2 levels, contributing to reprogramming
Onder et al.[47]	2012	Mice	Fibroblasts	epz004777	DOT1L inhibitor (Histone methyltransferase specific for H3K79)	With the reduction of DOT1L, a reduction in the methylation of H3K79 occurs, improving the reprogramming by increasing transcription factors such as Nanog and Lin28
Chen et al.[45]	2013	Mice	Fibroblasts	Vitamin C	Increases Kdm3 and Kdm4 – Lysine-specific demethylase	Reduces H3K9me2 and H3K9me3, important early in the reprogramming of iPSCs
Hou et al.[42]	2013	Mice	Fibroblasts	3-deazaneplanocin A (Dznep)	Histone methyltransferase inhibitor	Better reprogramming occurs with inhibition of histone methyltransferase by preventing methylation in the arginine and lysine residues of histones

OSKM: Oct4, Sox2, Klf4 and c-Myc; VPA/TSA/SAHA: valproic acid/trichostatin A/suberoylanilide hydroxamic acid; iPSCs: induced pluripotent stem cells.

TABLE 3 Epigenetic changes using microRNAs.

Authors	Year	miRNA	Species	Type of cell	Mechanism of action	Results
Judson et al.[54]	2009	290-295	Mice	Fibroblast	Blocks p21, leading to increased cell cycle proteins	Promotes the G1-S phase transition (proliferation), indirectly activating pluripotency factors
Li and He[50]	2012	Let-7	Mice	Embryonic stem cell	Reduced CyclinD/Cdk4 regulation	Blocks G1-S phase
Subramanyam et al.; Lin et al.[55,56]	2011	302-367	Mice	Fibroblast	Inhibits TGFII-β	Promotes MET
Choi et al.[63]	2011	34a and 34b/c	Mice	Fibroblast	Reduced regulation of p21 and p53	Indirectly blocks pluripotency factors
Li et al.[61]	2014	135b	Mice	Fibroblast	Reduced expression of TGF-β, Igfbp5, and Wisp1	Promotes MET
Zare et al.[49]	2015	124-128	Humans	Fibroblast	Regulate the development of neurons	Promotes the migration, maturation and differentiation of neurons, maintaining adequate gene expression and repressing unwanted genes

MET: mesenchymal-epithelial transition.

thology affects around 700,000 people in Japan, and is the most common form of blindness in people aged over 60, causing progressive loss of the retinal pigment epithelial monolayer. The transplant lasted around two hours and, according to the researchers, the patient did not suffer adverse effects, and there were an improvement in the morphology of the macula and neovascularization.[66]

This clinical trial was carried out by the group of Professor Takahashi, co-author of the manuscripts that won Professor Yamanaka the Nobel Prize in Medicine in 2012. However, in March 2015, clinical testing intended to treat six patients was suspended due to regulatory changes in regenerative medicine in Japan.[67]
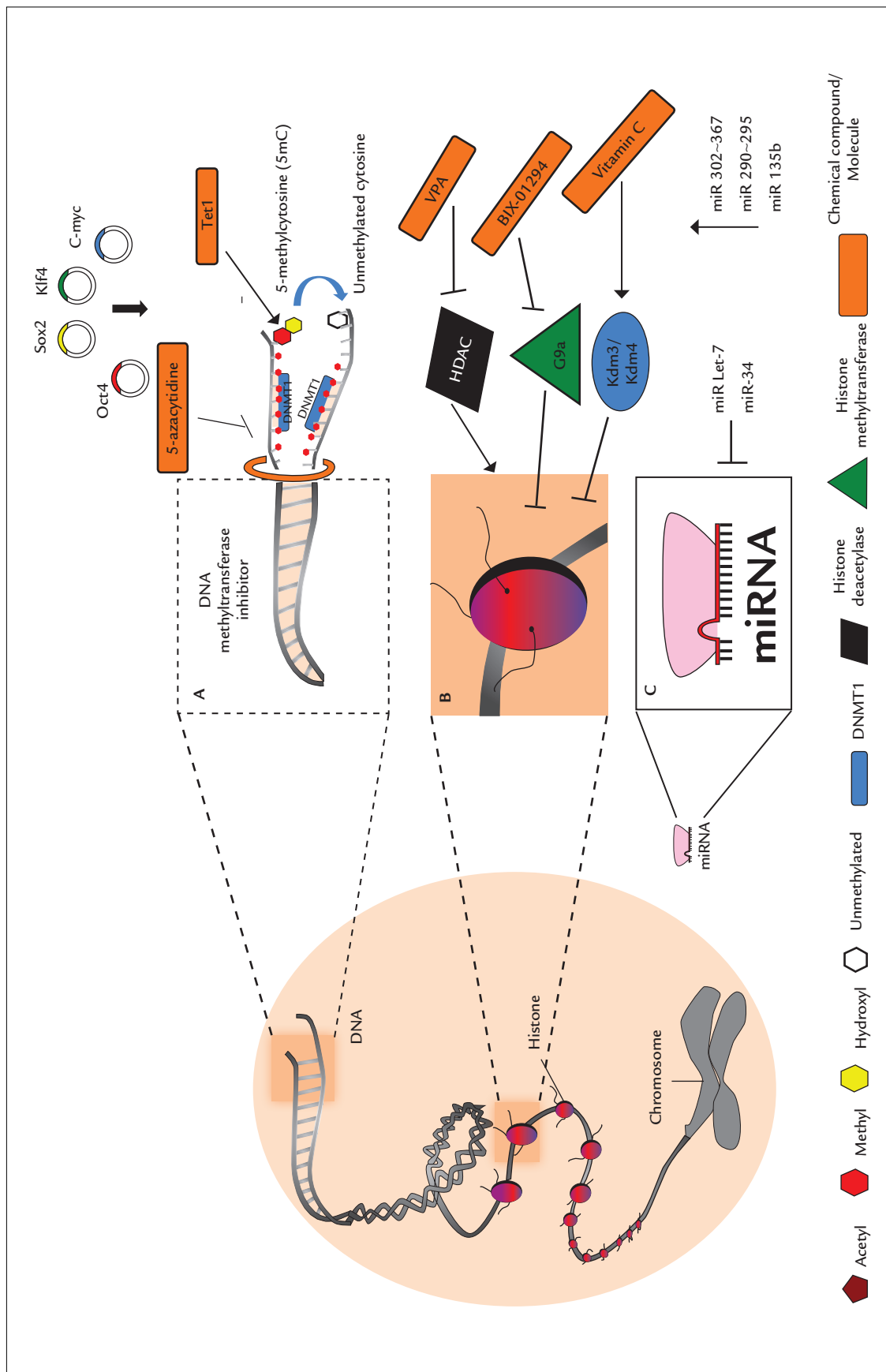
Other clinical trials are currently underway to test the effectiveness of cellular therapy with iPSC in the treatment of AMD, Parkinson's disease, spinal cord injury, diabetes and myocardial infarction.[68] Preliminary results have not yet been presented.
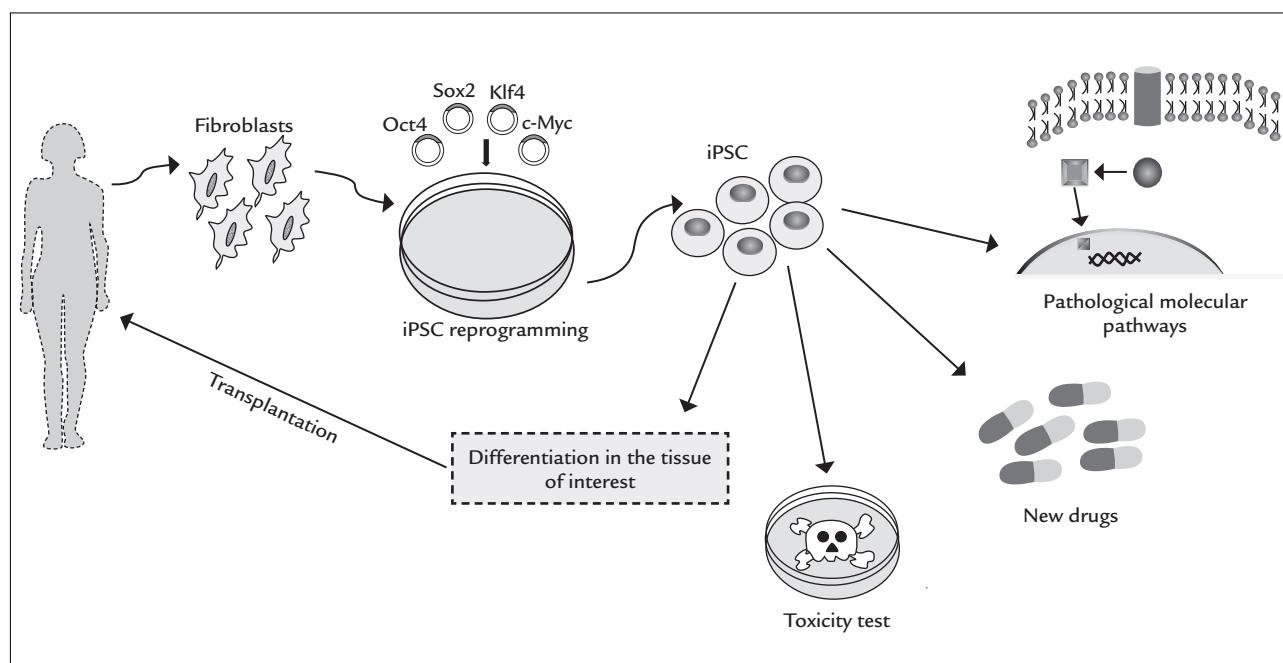
Maintenance of the epigenetic factors of the iPSC after reprogramming has also been used to understand the molecular pathways involved in the development of diseases, development of new drugs and personalized medicine.[69] The first study conducted of this kind used a model of neuropathic disease. The authors reprogrammed fibroblasts from patients with Riley-Day syndrome and monitored in vitro splicing of the IKBKAP (mutation associated with the disorder). Furthermore, the researchers also evaluated candidate drugs for reversal of the splicing. The study of iPSC gains relevance in this case, due to the inability of accessing the tissues

affected by Riley-Day syndrome.[70] Other studies have been developed along this line,[69] and are promising in the context of drug development. Figure 2 summarizes the use of iPSC.

Conclusion

Despite the improvement recently seen in iPSC reprogramming by up to 100 times, when compared to Yamanaka factors,[44] the yield remains relatively low and high costs. Another problem is the long period for full iPSC reprogramming and the high cells proliferation rates associated with a greater chance of developing cancer.[71] Takahashi and Yamanaka suggest considering using iPSC with allogeneic transplantation in regenerative medicine, which would improve the effectiveness of the treatment of certain diseases such as spinal cord injury, which requires quick treatment without waiting for the time taken for reprogramming.[11] Furthermore, there is a need for better understanding of how the reprogramming interventions influence the epigenetic memory of the reprogrammed cells. Despite advances in iPSC reprogramming, certain questions have emerged: 1) Is it possible to completely erase the somatic epigenetic memory by associating the different treatments mentioned?; 2) Could "forced" reprogramming cause long-term damage, such as the development of cancer or other diseases?; 3) Is it possible to replace embryonic stem cells with iPSC in regenerative medicine? In spite of the extraordinary progress achieved recently in the use of iPSC, the deepening of ongoing studies and realization of new studies are necessary in order to elucidate

FIGURE 1 Epigenetic factors and iPSC reprogramming efficiency. The ectopic expression of Yamanaka factors, Oct4, Sox2, Klf4 and c-Myc (OSKM) are able to lead to DNA demethylation and reprogramming of somatic cells. A. The use of Tet1 (DNA hydroxylase) and of 5-azacytidine (inhibitor of the enzyme DNA methyltransferase) improves the reprogramming efficiency of iPSC cells. B. The use of valproic acid (VPA), BIX-01294 and vitamin C favors reprogramming through inhibition of histone deacetylase (HDAC), histone methyltransferase (G9a) and activation of the demethylases Kdm3/Kdm4, respectively. C. miRNAs 302-367, 290-295, 135b are able to increase reprogramming efficiency by promoting the progression of the cell cycle. miR Let-7 inhibits the cell cycle, and miR-34 inhibits the translation of p53, decreasing reprogramming efficiency.

FIGURE 2 Somatic cells are reprogrammed into induced pluripotent stem cells (iPSCs). These cells are differentiated in the tissue of interest and transplanted in an attempt to reduce the damage caused by degenerative diseases. In addition, iPSCs are also being used in pre-clinical and clinical tests.

the mechanisms of the aforementioned changes, optimizing their application in regenerative medicine.

Resumo

Células-tronco de pluripotência induzida: papel da epigenética na reprogramação e sua aplicabilidade clínica

As células-tronco de pluripotência induzida (CTPI) ou do inglês *induced pluripotent stem cells* (iPSCs) são células somáticas reprogramadas para o estado embrionário por meio da expressão de fatores ectópicos de transcrição específicos, tornando-as um alvo promissor para a medicina regenerativa. Apesar das CTPI compartilharem características embrionárias, como pluripotência e capacidade de autorrenovação, elas possuem uma baixa eficiência de reprogramação, sendo a memória epigenética uma das principais barreiras nesse processo. A epigenética é caracterizada por alterações reversíveis e herdáveis no genoma funcional que não alteram a sequência de nucleotídeos do DNA. Dentre as diferentes modificações epigenéticas, destacam-se metilação de DNA, alterações em histonas e microRNA. Atualmente, sabe-se que o processo de reprogramação efetivo das CTPI envolve um completo remodelamento da memória epigenética somática existente, seguido pelo estabelecimento de uma "assinatura epigenética" que esteja de acordo com o novo tipo de célula a ser diferenciada. Modificações epigenéticas personalizadas são capazes de melhorar o rendimento e a efetividade das CTPI geradas, abrindo uma nova perspectiva para a terapia celular. Nesta revisão reunimos as principais informações sobre os fatores epigenéticos que afetam a reprogramação das CTPI, bem como seus benefícios na aplicação da terapia celular.

Palavras-chave: células-tronco de pluripotência induzida, medicina regenerativa, reprogramação celular, epigenética, histonas, microRNA.

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